Comment on USDA’s TB & Brucellosis Program Changes

On December 16, 2015, the USDA-Animal and Plant Health Inspection Service (APHIS) published a Proposed Rule, Brucellosis and Bovine Tuberculosis; Update of General Provisions, regarding changes in the regulations for the bovine tuberculosis (bTB) and brucellosis programs. The public comment period is open through March 15, 2016.

The following is excerpted from the notification in the Federal Register: “We are proposing to consolidate the regulations governing bovine tuberculosis, and those governing brucellosis. As part of this consolidation, we are proposing to transition the tuberculosis and brucellosis programs away from a State classification system based in disease prevalence. Instead, States and Tribes would implement animal health plans that identify sources of the diseases within the State or Tribal lands and specify mitigations to address the risk posed by those sources. The consolidated regulations would also set forth standards for surveillance, epidemiological investigations, and affected herd management that must be incorporated into each animal health plan, with certain limited exceptions; would provide revised conditions for the interstate movement of cattle, bison, and captive cervids…”

In stating the need for the proposed regulatory changes, USDA-APHIS cited two major factors that have developed and are impeding their cooperative efforts with States, Tribes, producers, and others to eradicate bTB and brucellosis. The first is the identification of wildlife reservoirs of these diseases in selected areas: Free-ranging white-tailed deer are the reservoir of bTB in the northeastern portion of Michigan’s Lower Peninsula, and wild bison and elk are reservoirs of brucellosis in the Greater Yellowstone Area. These are the only two areas in the U.S. in which native wildlife is involved in the epidemiology of bTB and brucellosis. The second factor is the substantial change in the cattle industry since the bTB and brucellosis regulatory programs were established early in the 20th century. Producers increasingly rely on imported cattle to supplement their herds, exposing the U.S. cattle herd to animals from regions with variable disease risk levels. In addition, herd sizes have increased substantially, market channels have become more complex, and interstate movements of captive cervids and bison have increased. The industry changes, along with decreased availability of Federal funds, have made it difficult or impractical to indemnify owners when whole-herd depopulation is employed to deal with infected cattle. Consequently, APHIS will determine if a test-and-remove or depopulation management plan will be supported with Federal indemnity for each brucellosis- or bTB-affected herd of program animals (cattle, captive cervids, and bison) based upon each herd’s unique circumstances.

The bTB and brucellosis classification of a State or Tribe would be based on animal health plans that address these diseases, APHIS approval of the plans, and whether the plans have been implemented and maintained. Selected items related to wildlife that are required in the animal health plans include a description of the organization and infrastructure of the animal health and wildlife authorities in the State or Tribe, including the workforce of wildlife biologists and veterinarians available to conduct disease mitigation measures listed in the plan and the individual designated to oversee implementation, performance, and enforcement of wildlife activities.
A description of the known sources of bTB or brucellosis, such as infected wildlife populations, that pose a risk of disease introduction to program animals is required, including a risk assessment of the likelihood of disease transmission. The description and assessment would include the numbers of wildlife populations and animals in them; the approximate prevalence of bTB or brucellosis in the populations, their geographic distribution, and any other factors that make the populations a potential source of transmission to program animals. This information would be obtained by conducting “...surveillance of that source population in a manner approved by APHIS as sufficient to detect brucellosis or tuberculosis in an animal within the source population.” Also required would be an evaluation of the potential for exposure of program animals to the known sources, and factors other than the mitigation measures that would be implemented, such as the movement patterns of animals and seasonal variations in potential exposure, that influence potential exposure and transmission of bTB or brucellosis to program animals.

The Proposed Rule and supporting documents, including the accompanying Brucellosis and Bovine Tuberculosis Program Standards, can be accessed and comments on the rule can be submitted electronically through: http://www.regulations.gov/#!docketDetail;D=APHIS-2011-0044. Written comments can be submitted to Docket No. APHIS-2011-0044, Regulatory Analysis and Development, PPD, APHIS, Station 3A-03.8, 4700 River Road Unit 118, Riverdale, MD 20737-1238. (Prepared by John Fischer)

Deer Malarial Parasite Rediscovered

*Plasmodium odocoilei*, a blood parasite of white-tailed deer that previously had been detected in just a single deer in 1967, recently has been shown to be common in deer throughout the eastern United States. Researchers at the Smithsonian Conservation Biology Institute and collaborators were conducting research on malarial parasites (*Plasmodium* spp.) in mosquitoes in order to better understand the epidemiology of avian malaria. Several of the mosquitoes tested positive for *Plasmodium*, but genetic sequencing showed the parasites were more similar to mammalian malarial species than to avian malarial parasites. The researchers then identified the blood in the mosquito abdomens to be of white-tailed deer origin. With that finding and subsequent sampling, white-tailed deer were identified as a common host in the area of Virginia where the mosquitoes had been trapped. Results of the study recently were published in *Science Advances*, which is available free online at: http://advances.sciencemag.org/content/2/2/e1501486.full.

Worldwide, malaria is a significant and sometimes fatal protozoal disease of humans. It is caused by five *Plasmodium* species, with *P. falciparum* causing most of the fatalities. Human malaria is transmitted most frequently by female *Anopheles* mosquitoes. There is only one known zoonotic *Plasmodium* species: *P. knowlesi*, which occurs in nonhuman primates in Southeast Asia and can cause clinical malaria in people. *Plasmodium odocoilei* is not known to be transmissible to humans, and there is no indication that this parasite represents a public health risk.

Thanks to years of SCWDS research on white-tailed deer conducted with the assistance of our cooperators, we were well positioned to provide a bank of white-tailed deer spleens for testing. Smithsonian researchers also were provided with white-tailed deer samples from the eastern United States as well as samples from elk, mule deer, black-tailed deer, and pronghorn from the western U.S. by SCWDS and other collaborators. More than 300 deer were tested and positive animals were identified from ten eastern states as far north as New York. Overall, 25% of the deer tested were positive, but at some sites, the prevalence reached 40-60%. No infections were detected in any other species, nor in white-tailed deer west of Louisiana. Interestingly, no deer from Texas tested positive even though the parasite originally was found there 50 years earlier.

This malarial protozoan had been reported in an adult deer from Texas that had been splenectomized as part of a blood pathogen study. Parasitemia first appeared eight days
after splenectomy and persisted for at least 24 days. The maximum number of parasites observed remained very low (only 1 in 30,000 red blood cells were infected) and the deer remained clinically normal without developing anemia. The parasite later was described as P. odocoilei by Drs. Garnham and Kuttler.

So, how did this parasite go undetected for so long? The original case was found only after a deer was splenectomized, and very few red cells were infected. The deer from Virginia that tested positive on blood smear analysis in the current study also had very few infected red cells. The detection of P. odocoilei in deer in numerous states was accomplished by molecular analysis of blood and tissue samples rather than by visual observation in blood smears. Molecular analysis for Plasmodium is more sensitive than blood smear examination and had not been conducted in deer prior to this study.

What impact does this parasite have on the health of white-tailed deer? The easy answer is that we do not know; however, this parasite is common and geographically widespread in whitetails in the eastern United States and clinically affected deer have not been detected. However, clinical malaria is difficult to diagnose and wildlife mortalities are easy to miss. Additionally, the subclinical effects of parasites on wildlife are difficult to study and understand. For example, a long-term study of avian malaria, which is caused by infection with P. relictum, in warblers in Europe found that chronic subclinical infection shortened the life-span of the birds. In addition, molecular testing of P. odocoilei suggests that it is millions of years old and may have a long co-evolutionary history with deer. Although it appears unlikely that this parasite has management implications for deer, future studies are necessary to evaluate the potential impacts of this parasite on deer health. (Prepared by Michael Yabsley)

**WHO Declares Zika Virus Public Health Emergency**

**NOTE:** Although there currently is no known wildlife involvement in the epidemiology of Zika virus in the Americas, the following information is provided for those of you who spend time outdoors and are vulnerable to infection with insect-borne pathogens.

On February 1, 2016, the World Health Organization (WHO) announced a Public Health Emergency of International Concern and called for a rapid, coordinated response to the spread of Zika virus. The virus first was isolated in 1947 from a rhesus macaque in Zika forest, Uganda, and only 14 sporadic human infections were reported within a narrow equatorial belt from Africa to Asia over the following 60 years.

Things began to change in 2007 when Zika virus infected 70% of the human population on Yap Island, Federated States of Micronesia, and caused clinical disease. Another large outbreak of Zika virus disease was identified in 2013 in French Polynesia, with more infections reported in 2014 on Easter Island (Chile) and in 2015 in Brazil, where the number of cases has soared since last February. Since October 2015, Zika virus has spread throughout northern-central South America, Central America, the Caribbean, and into Mexico, and public health officials are concerned that athletes and spectators attending the Olympic Games in Brazil this summer may facilitate the global spread of the virus.

Zika virus disease occurs in approximately 20% of infected persons, as is seen with West Nile virus infections. Clinically affected patients may have mild fever, rash, muscle and joint pain, headache, and conjunctivitis that usually subsides within a week. However, there are growing concerns over the possible association of Zika virus with severe birth defects, such as microcephaly, in babies born to women infected during pregnancy, as well as with Guillain-Barré Syndrome (GBS), an autoimmune disease in which the immune system attacks the nervous system, sometimes resulting in respiratory paralysis. Brazil has seen a significant increase in babies born with microcephaly in areas with Zika virus transmission, and viral genetic material has been detected in amniotic fluid of women carrying fetuses with microcephaly as well as in tissues of one dead newborn with the defect. The potential role of Zika virus infection...
in birth defects, GBS, and other conditions continues to be investigated.

Zika virus is in the genus *Flavivirus*, which includes the viruses that cause dengue, West Nile, and yellow fevers. Zika virus primarily is transmitted by mosquitoes in the genus *Aedes*, especially *A. aegypti* and *A. albopictus*, which also can serve as vectors of chikungunya virus and the dengue and yellow fever viruses. These mosquitoes are closely associated with humans and are widely distributed throughout the Americas, including parts of the United States. Zika virus transmission also has been documented from mother to child at the time of delivery, or possibly during gestation, as well as through blood transfusion and sexual contact. Transmission modes other than insect bites also are being investigated.

Public health officials at the U.S. Centers for Disease Control and Prevention (CDC) suggest that outbreaks of Zika virus are inevitable in the U.S., but predict they will be limited in scope. To date, Zika virus infection has been detected in 52 persons in the U.S. that had visited areas where outbreaks are occurring, and the State Surgeon General and the Secretary of Health in Florida have declared a Public Health Emergency in the eight counties in which 20 travel-associated cases have occurred. Until recently there was no evidence that any of the recent infections were acquired in the U.S.; however, in early February the Dallas County Health and Human Services in Texas announced that CDC had confirmed a patient became infected after having sexual contact with an individual who returned from a country where Zika virus transmission is occurring.

There is no vaccine for Zika virus. Public health officials advise travelers, especially pregnant women, to consult public health advisories regarding the areas they will visit in order to identify regions of Zika virus transmission and preventive measures to be taken, including possible postponement of travel. The best way to prevent Zika virus infection is mosquito bite avoidance, which also prevents transmission of several other mosquito-borne pathogens. The CDC currently is developing additional guidelines including recommendations to prevent sexual transmission with a focus on male sexual partners of women who are or who may become pregnant. The following CDC websites provide more information on Zika virus and insect bite prevention:


(Prepared by Danny Mead)

**Severe Larval Fluke Infection in a Tree Frog**

In December 2015, an adult, female green tree frog (*Hyla cinerea*) with unusual skin lesions was found at a water treatment facility in Chatham County, Georgia. The frog was brought into captivity but died 33 days later. The carcass was submitted on behalf of the Georgia Department of Natural Resources to SCWDS for diagnostic evaluation.

The frog was emaciated and had no visible fat stores. The skin was covered with approximately 300 raised, uniform, tan-green subcutaneous nodules approximately 2-3 mm in diameter, occasionally with central regions of depression (Figure 1).

![Figure 1.](image)

Each nodule contained one to three larval flukes (trematodes) that were 3-4 mm long that were identified as *Clinostomum* spp. metacercariae. Microscopically, the nodules consisted of cyst-like structures surrounded by a clear space and a ring of fibrous connective tissue that
occasionally was infiltrated by small numbers of inflammatory cells. The parasitic nodules were present throughout the skin and occupied the majority of all skin sections that were examined microscopically. The nodules also were present throughout the coelom and were embedded within the oral cavity, orbit, calvarium, adrenal gland, kidney, liver, lung, and ovary. Increased numbers of melanomacrophages were observed in the liver and kidney, indicating a chronic inflammatory response, and mild degenerative changes were apparent in the liver.

Clinostomum spp. are digenean flukes with an indirect life cycle requiring more than one host for completion. The first required intermediate host is an aquatic mollusk. Because of this, the prevalence of these parasites can vary greatly based on habitat type and/or the period of time in which a wetland is covered with water. Although little is known about Clinostomum spp. life cycles in amphibians, related species of Clinostomum that infect fish have been studied more extensively because they cause unsightly "yellow grub" lesions in fish raised commercially for human consumption. Typically, amphibians and fish are infected via skin penetration by swimming larvae (cercariae) released from infected snails. Adult Clinostomum spp. flukes are found in the oral cavity and esophagus of numerous water bird species such as herons and egrets.

Although rare, Clinostomum infection of free-ranging amphibians has been reported. However, the present case was unusual because most infected frogs have very low parasite burdens. Although the ultimate cause of death in this case likely was starvation due to inappetence, the severe parasitic infection would have contributed significantly to this frog's demise. In addition, the larval flukes in multiple organs in this frog may have interfered with normal organ function and contributed to overall poor health. Infection with this parasite can decrease survival of infected individuals, and this case likely represents a singular event without population-level impacts. Another digenean trematode that can be found encysted in amphibians is Ribeiroia ondatrae, an important cause of limb deformities. To date, limb deformities in amphibians have not been associated with Clinostomum spp. (Prepared by Heather Fenton and Michael Yabsley)

Comment on Proposed Changes to Select Agent List

On January 19, 2016, the Federal Select Agent Program (FSAP) announced that they are considering revising the current list of select agents and toxins by removing several agents and making other changes. Those under consideration for removal are Coxiella burnetii, Rickettsia prowazekii, Bacillus anthracis Pasteur strain, Brucella abortus and B. suis, Peronosclerospora philippinensis, Phoma glycinicola, and Sclerophthora rayssiae. The Select Agent Division within the U.S. Centers for Disease Control and Prevention (CDC) also recommends removal of Brucella melitensis, but the USDA Animal and Plant Health Inspection Service (APHIS) Select Agent Division proposes to keep it on the list because it is considered a foreign animal disease agent.

The select agent and toxin list is reviewed at least every two years to determine if agents or toxins should be added to or deleted from the list. The CDC and USDA-APHIS have published separate notices in the Federal Register regarding the proposed changes. The notices are available online:


The FSAP was established to satisfy requirements of the U.S.A. Patriot Act of 2001, the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, and the Agricultural Bioterrorism Act of 2002. The two divisions of the FSAP within the CDC and USDA-APHIS work together to oversee the possession, use, and transfer of biological select agents and toxins, which currently number 65. Select agents and toxins are those that have been identified as a severe threat to human and
domestic animal health as well as to animal or plant products. The USDA-APHIS has authority over agents and toxins that are a threat to animals and plants and their products; the CDC has authority over those agents that are deemed a threat to humans, and both agencies share authority for agents on the overlap list that threaten humans and domestic animals.

The select agent regulations, which were intended to benefit society by restricting access to certain pathogens and toxins, also created a framework for prosecuting individuals not properly registered for using the listed agents. In a nutshell, the purpose of the regulations was to mitigate human and domestic animal health risks by imposing a strict regulatory environment for individuals working with select agents and toxins. Although successful, some felt the FSAP hindered or prohibited research into preventive measures and therapeutics, as well as diagnostic test development, for select agents. For example: a 2004-05 survey of select agent users in the U.S. found that over 20% of them felt that FSAP regulations affected their ability to collaborate domestically and internationally, and roughly 40% of them had to use research funding to make required security upgrades. One example of interest to wildlife and livestock stakeholders is Brucella abortus, which is endemic in wild elk and bison in the Greater Yellowstone Area and spills over into domestic cattle sporadically. Some believe brucellosis research has been hindered by the FSAP, and it is hoped that deletion of B. abortus from the list will expand opportunities for development of effective vaccines.

The FSAP invites interested persons and organizations to participate in the review process and rulemaking and is accepting written comments, views, and recommendations until March 21, 2016. Comments identified by Docket numbers CDC-2015-0006 or RIN 0920-AA can be submitted to the CDC by mail (Division of Select Agents and Toxins, Centers for Disease Control and Prevention, 1600 Clifton Road NE., MS-A46, Atlanta, Georgia 30329, Attn: Docket CDC-2015-0006) or electronically (http://www.regulations.gov/#/documentDetail;D=CDC-2015-0006-0024). Comments, views, and recommendations to USDA-APHIS can be submitted by mail (Docket No. APHIS-2014-0095, Regulatory Analysis and Development, PPD, APHIS, Station 3A-03.8, 4700 River Road Unit 118, Riverdale, Maryland 20737-1238) or electronically at http://www.regulations.gov/#/docketDetail;D=APHIS-2014-0095.

(Prepared by Danny Mead.)

Armadillos and Leprosy

Researchers from the National Hansen’s Disease Program at Louisiana State University (LSU) and their collaborators recently published the results of a study that found armadillos (Dasypus novemcinctus) infected with Mycobacterium leprae in eight locations in Alabama, Florida, Georgia, and Mississippi. Historically, natural infections of free-ranging armadillos with M. leprae were reported only in Texas and Louisiana, and a large field survey conducted by SCWDS in the early 1990s had failed to find infected armadillos in Alabama, Florida, and Georgia. The LSU researchers also found an association between M. leprae strains in naturally infected armadillos and in human patients. Their study was published in December 2015 in Emerging Infectious Diseases (http://wwwnc.cdc.gov/eid/article/21/12/15-0501_article).

Leprosy, now known as Hansen’s disease, is a chronic infectious disease of humans that primarily affects the skin and nerves and is caused by the bacterium M. leprae. Due to the severe disfigurement that can occur in untreated chronic infections, patients historically were considered social outcasts. Most transmission occurs via aerosols or direct contact between humans. Although Hansen’s disease is mainly a concern in parts of Africa, Asia, and South America, about 200 cases are diagnosed in the United States every year. Many of these cases are associated with travel to endemic countries, but recent studies indicate that some infections were acquired in the U.S., and many were associated with exposure to infected armadillos.

The range of nine-banded armadillos once was restricted to Texas and Louisiana; however, it has expanded greatly in recent decades and armadillos now are found as far north as Illinois. They are highly susceptible to M. leprae, can
develop high bacteria loads and clinical disease, and have been used as a laboratory animal model to understand the disease in people.

In the recently published study, the role of armadillos as potential reservoirs of *M. leprae* infecting humans was investigated by genetic characterization of *M. leprae* strains from 52 human cases and 42 infected armadillos. Two strains of *M. leprae* were found in infected armadillos in the U.S. One strain, which had not been observed previously in armadillos, occurred only in southern Florida in 7/42 (17%) infected armadillos and 10 human patients, while the predominant strain was found in 35/42 (83%) of infected armadillos from Alabama, Georgia, northern Florida and Mississippi, and in 12 human cases, all of which occurred in areas in the southern U.S. where they may have been exposed through armadillos. Overall, 42% of human patients were infected with *M. leprae* genotypes associated with armadillos.

The state of Florida experienced a dramatic increase in reported cases of Hansen’s disease in 2015 according to the Florida Department of Health. The number of cases ranged from 10-12 annually during the previous five years; however, 27 cases were reported in 2015, and five more cases have been reported to date in 2016.

Several epidemiologic studies indicate that armadillo contact is a risk factor for acquiring *M. leprae* infections in the U.S., although many patients do not recall having exposure to armadillos. Therefore, other transmission routes, such as exposure to bacteria in the environment, have been suggested and highlight the need for more data to fully understand risk factors associated with transmission. As with all wildlife species, only trained professionals should handle animals, proper protective equipment, such as gloves, should be worn, and strict hygienic practices should be observed to minimize the risk of transmission of zoonotic pathogens. (Prepared by Sarah Leyman from The Ohio State University and Michael Yabsley)

### New Diseases of Feral Swine Brochure

A brochure, *Diseases of Feral Swine*, recently was developed and published by SCWDS and the USDA-APHIS-Wildlife Services (WS) National Feral Swine Damage Management Program. The brochure contains current information on a number of foreign and domestic pathogens that may occur in feral swine and is a user-friendly reference for people working with these animals.

Feral swine serve as hosts, carriers, and/or dispersers of pathogens that can significantly impact domestic animal and human health as well as wildlife health. *Diseases of Feral Swine* is organized in a straightforward manner by pathogen group with distinct, color-coded sections dedicated to bacterial, parasitic, and viral diseases. Each pathogen is described through the answers to four basic questions: What is it? How is it transmitted? Can it be transmitted to domestic swine and other livestock? Can it be transmitted to humans? This simplified format keeps the focus on key epidemiological facts such as transmission modes, host range, and zoonotic potential.

*Diseases of Feral Swine* is accessible in multiple platforms: Hardcopies can be obtained from USDA-APHIS-WS (866-487-3297) or SCWDS (706-542-1741), and the electronic file is available on the SCWDS website:


In addition, a smartphone software application is in development, and all three formats of the brochure soon will be available in Spanish. (Prepared by John Bryan)